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14. ABSTRACT
Hemorrhage is a leading cause of deaths on the battlefield. An understanding of the mechanisms and modulators of coagulopathy under conditions soldiers currently experience on the battlefield is important for improved treatment of the hemorrhaging soldier. The global objective of this project tests the hypothesis that environmental and physiological conditions a soldier experiences on the battlefield alters hemodynamic and hemostatic function (i.e., coagulation and fibrinolysis). During the current funding period, we sought to obtain the required IRB and ORP approvals, acquire the necessary equipment to accomplish the stated aims, and complete the objectives in specific aim 1. Aim 1 tests the hypothesis that passive heat stress alters hemostatic function during simulated hemorrhage. ORP documents were submitted for initial administrative review April 4, 2012 and final approval was granted October 10, 2012. Data collection began November 7, 2012. Each subject completes a normothermic and heat stress trial (both including a hemorrhagic challenge), separated by at least 60 days (owing to the amount of blood drawn for each trial). 10 subjects have completed both trials, with the final 2 subjects completing their second trial this May. Data from blood based assays of hemostatic function are presently being processed, while plasma-based assays are expected to be run by Dr. Cap's laboratory at the US Army Institute of Surgical Research by August 2013.

15. SUBJECT TERMS
hemorrhage, hyperthermia, heat stress, coagulopathy, hemostasis.

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Table of Contents

Introduction

Worldwide, trauma is the cause of 1 in 10 deaths, with 30-40% of trauma deaths due to hemorrhage. Hemorrhage is also a leading cause of death on the battlefield. An understanding of the mechanisms and modulators of coagulopathy under conditions soldiers often experience on the battlefield is important for improved medical treatment. The global objective of this project is to test the hypothesis that environmental and physiological conditions a soldier experiences on the battlefield alters hemodynamic and hemostatic function (i.e., coagulation and fibrinolysis) resulting in compromised ability to survive a hemorrhagic injury. This objective will be accomplished by evaluating the following Specific Aims: 1) Passive heat stress alters hemostatic function during simulated hemorrhage. 2) Dehydration during exercise-induced hyperthermia alters hemostatic function during a subsequent simulated hemorrhage. 3) Heating a hemorrhaging individual who is not hypothermic is detrimental to blood pressure control, cerebral perfusion, and hemostatic function. This project will provide the Department of Defense with valuable information resulting in improved medical treatment of soldiers who have experienced a hemorrhagic injury while in hyperthermic environmental conditions.

Body

The global objective of this project tests the hypothesis that environmental and physiological conditions, that a soldier often experiences on the battlefield, alters hemodynamic and hemostatic function (i.e., coagulation and fibrinolysis). The following contains the items under the Statement of Work that we proposed would be accomplished during Year 1 of the project:

1) Obtain IRB/ORP approvals from the University of Texas Southwestern Medical Center, Texas Health Presbyterian Hospital Dallas, and the Department of Defense for the proposed studies.
2) Purchase the TEG5000 units and become proficient in their use.
3) Accomplish objectives outlined in Specific Aim 1A. This study will investigate the effects of passive heat stress on markers of hemostatic function during simulated hemorrhage.
4) Analyze and interpret data obtained during the experiments outlined in Specific Aim 1A.

Upon notification of funding, we obtained IRB approvals from the University of Texas Southwestern Medical Center and Texas Health Presbyterian Hospital Dallas. ORP documents were submitted in early April 2012, and following a number of revisions final ORP approval was granted October 10, 2012. During this time we acquired the TEG5000 units and became proficient in their use. With both IRB and ORP approval in hand, and with us being competent in the TEG5000 assays, we began data collection on November 7, 2012. Given delays associated with ORP approval, we have been collecting data for ~5 months. Despite this relatively short period of time, we have done an outstanding job towards completing the objectives of Aim 1.

Aim 1 tests the specific hypothesis that passive heat stress alters hemostatic function during simulated hemorrhage. The protocol requires 12 subjects to visit the laboratory on two occasions, separated no fewer than 60 days, owing to the amount of blood

drawn for each trial relative to the duration needed for the body to replenish that blood. During one visit the subjects are passively heat stressed sufficient to increase internal temperature ~1.2 °C. Upon achieving this temperature, subjects are exposed to progressive simulated hemorrhage via lower-body negative pressure (LBNP) until the onset of syncopal symptoms. During the other visit, subjects remain normothermic for a comparable time relative to the aforementioned heat stress trial, after which they undergo the same LBNP challenge. The order of exposure to these challenges is randomized. Ten subjects have completed both limbs of the experiment, with the final 2 subjects scheduled to complete their trials in early May. Thus, the data presented herein are from the 10 subjects who completed both limbs of the protocol.

By design, heat stress increased internal temperature prior to LBNP by 1.22±0.05 °C. Tolerance to graded LBNP was reduced by ~50% when subjects were heat stressed (8.3±4.1 min; P<0.01) relative to normothermic (16.8±7.9 min). The following data were obtained at normothermic baseline, during heating or normothermic time control just prior to LBNP, throughout LBNP inclusive of presyncope, and during recovery:

<u>Flashback Technologies:</u>
Pulse oximetry derived predictions of Compensatory Reserve Index (see Figure 1).

<u>Reflectance Medical:</u>
Forearm near-infrared spectroscopy signals

<u>Body Media:</u>
Activity level, local skin temperature, and heart rate

Data from each of these sensors will be processed by the respective companies. Each of these companies either currently has, or previously had, DOD funding to process these signals during simulated hemorrhage in normothermic subjects. The unique

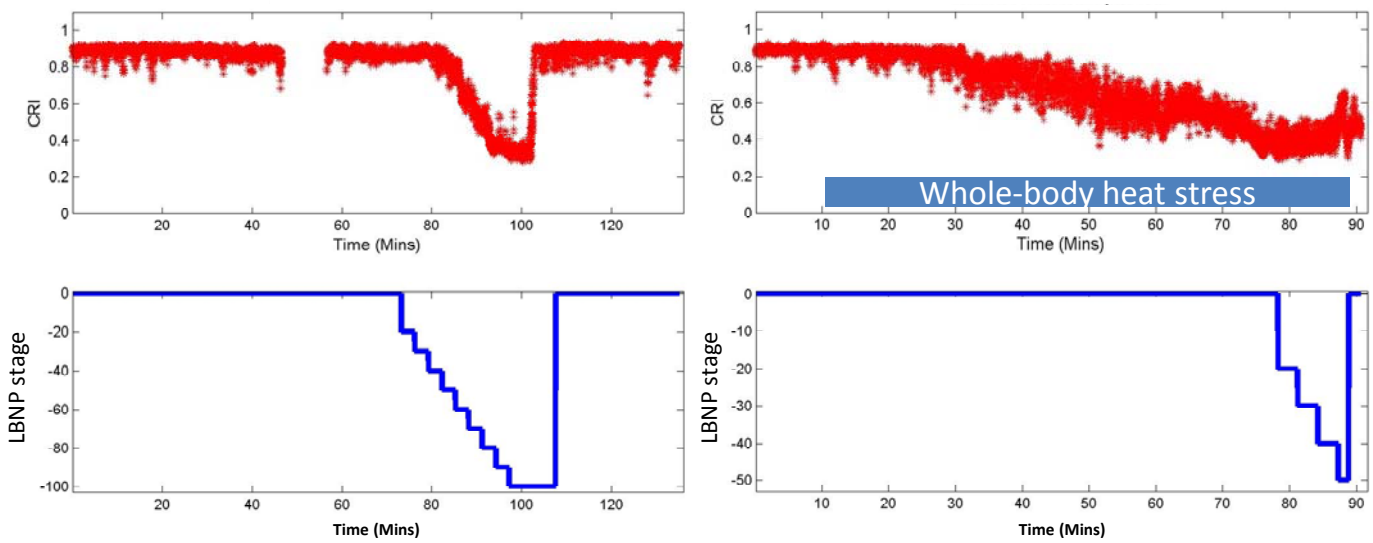

Figure 1: Normothermic (left panel) and heat stress (right panel) data of the Compensatory Reserve Index (CRI; from Flashback Technologies) at baseline and during lower-body negative pressure (LBNP) to pre-syncope. LBNP is indicated by the blue step-down figure. For this subject, CRI is affected by heat stress independent of LBNP. Moreover, heat stress alters CRI responses during LBNP. However, these data are very preliminary and need to be confirmed with all 12 subjects.

aspect of this study is the evaluation of these devices to predict hemorrhagic status in heat stressed (and ultimately dehydrated) individuals. We have preliminary results of a trial from Flashback Technologies (see figures below). Final results from these devices are forthcoming from the indicated companies upon completion of data collection from all 12 subjects.

The combined effect of heat stress and simulated hemorrhage on markers of blood coagulation is another primary measure from this protocol. For both normothermic and heat stress trials, blood is obtained at the time points indicated by the arrows in Figure 2.

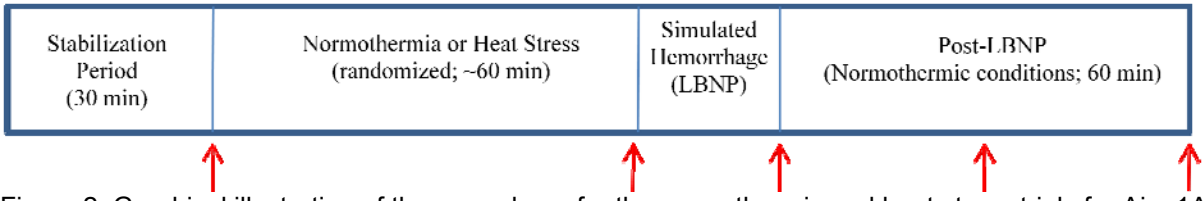

Figure 2: Graphical illustration of the procedures for the normothermic and heat stress trials for Aim 1A. The arrows indicate when blood is withdrawn for evaluation of markers of hemostatic function. Thermal and hemodynamic variables will be continuously obtained. LBNP: lower body negative pressure.

For plasma based assays, the blood is immediately centrifuged and plasma is extracted and frozen in a -80 °C freezer. Upon completion of data collection from all 12 subjects, the frozen plasma will be shipped to the United States Army Institute for Surgical Research (USAISR) for analysis of the following hemostatic parameters:

Prothrombin Time (PT): Evaluates the integrity of the extrinsic coagulation pathway.
Activated Partial Thromboplastin Time (aPTT): Evaluates the integrity of the intrinsic coagulation pathway.
D-Dimer: A marker of fibrinolysis.
Fibrinogen: Protein that thrombin converts to fibrin during the clotting process.
Tissue Plasminogen Activator (tPA): A protein the contributes to the breakdown of clots through catalyzing the conversion of plasminogen to plasmin.
Antithrombin III: Inactivates enzymes facilitating coagulation (factors Xa and IIa).
Protein C: An anticoagulant that contributes to the regulation of clotting by inactivating factors V and VIII, as well as by disinhibiting fibrinolysis through inactivation of PAI-1 (see below).
Plasminogen activator inhibitor-1 (PAI-1): Inhibits tissue plasminogen activator (tPA, see above), thereby inhibiting fibrinolysis.
Von Willerand factor (vWF) antigen: A measure of the quantity or mass of Von Willerand protein in plasma, which contributes to the early stages of clotting.
Factors V and VIII: Factors essential in blood coagulation. Factor V is a critical cofactor for factor X which in turn activates factor II to IIa (Thrombin). Factor VIII is a critical cofactor for the factor IX-medicated activation of factor X.

Because we have not completed data collection in all 12 subjects, these samples have not been transported to USAISR and thus we do not know the outcome of the applied perturbations on these markers of hemostatic function.

However, whole blood-based measures of hemostatic function are obtained “at bedside” immediately after each draw. These data are obtained via thromboelastrography from the TEG5000 units (see Figure 3). The following four parameters were evaluated from this assay, Reaction Time (R): the amount of time until initial clotting; Kinetic time (K) and α Angle: measures of the rate of clot growth; Maximum Amplitude (MA): Maximal clot strength; and percent lysis of the clot 30 min after MA (LY30).

Data from these assays were analyzed via a two-way repeated measures ANOVA with main factors of thermal condition (normothermia and heat stress) and stage (see arrows in Figure 2). For each of the evaluated variables, there was a significant effect of stage but not a significant effect of thermal condition nor a significant interaction (see Figures 4-7). Although the applied conditions clearly alter hemostatic function, the magnitude of the responses were unaffected by heat stress. Data for LY30 are still under analysis.

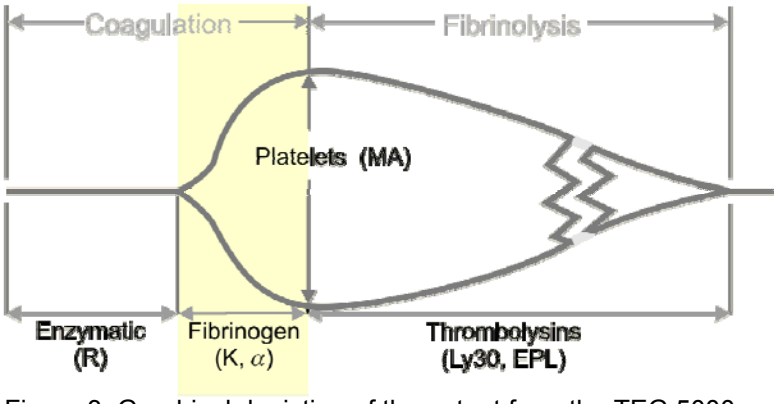

Figure 3: Graphical depiction of the output from the TEG 5000 thermoelastograph analyzer. Multiple variables are obtained from this computer-based analysis system pertaining to blood coagulation and fibrinolysis potential. R: Reaction time; K: Kinetic time; α: Alpha angle; MA: Maximum amplitude. Figure from Haemonetics.

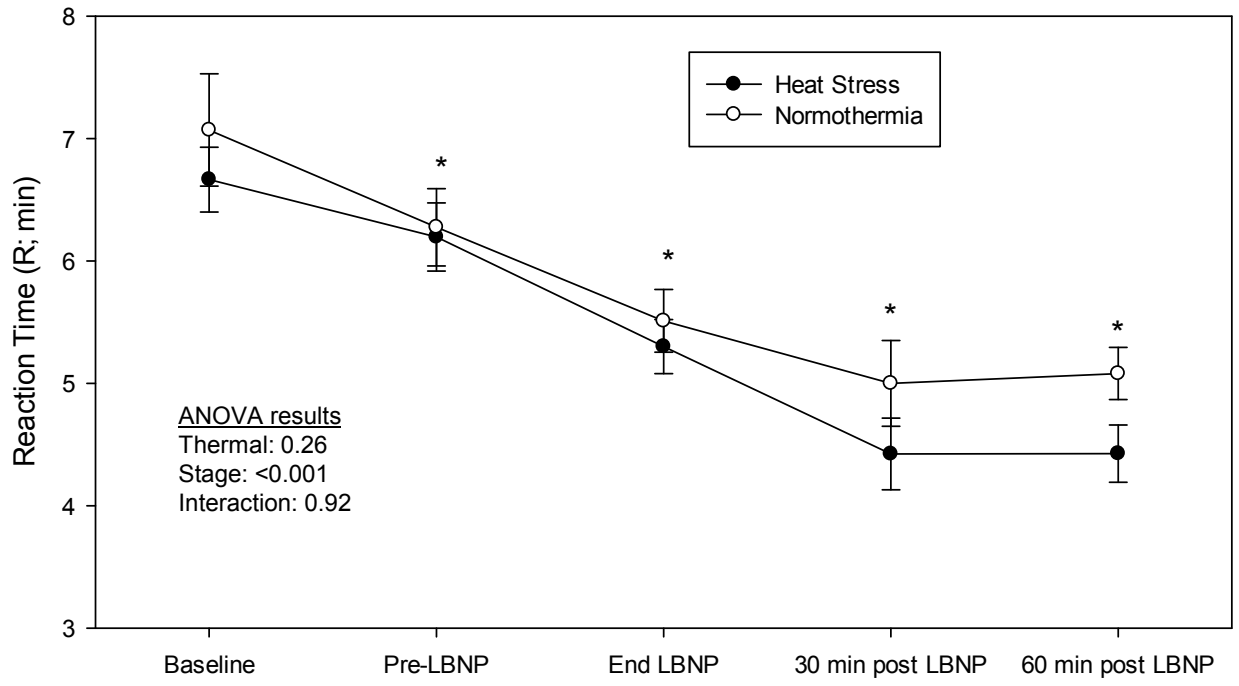

Figure 4: Effect of heat stress and lower-body negative pressure (LBNP) on thromboelastography reaction time (R). * significant difference for the indicated stage relative to baseline, regardless of thermal condition.

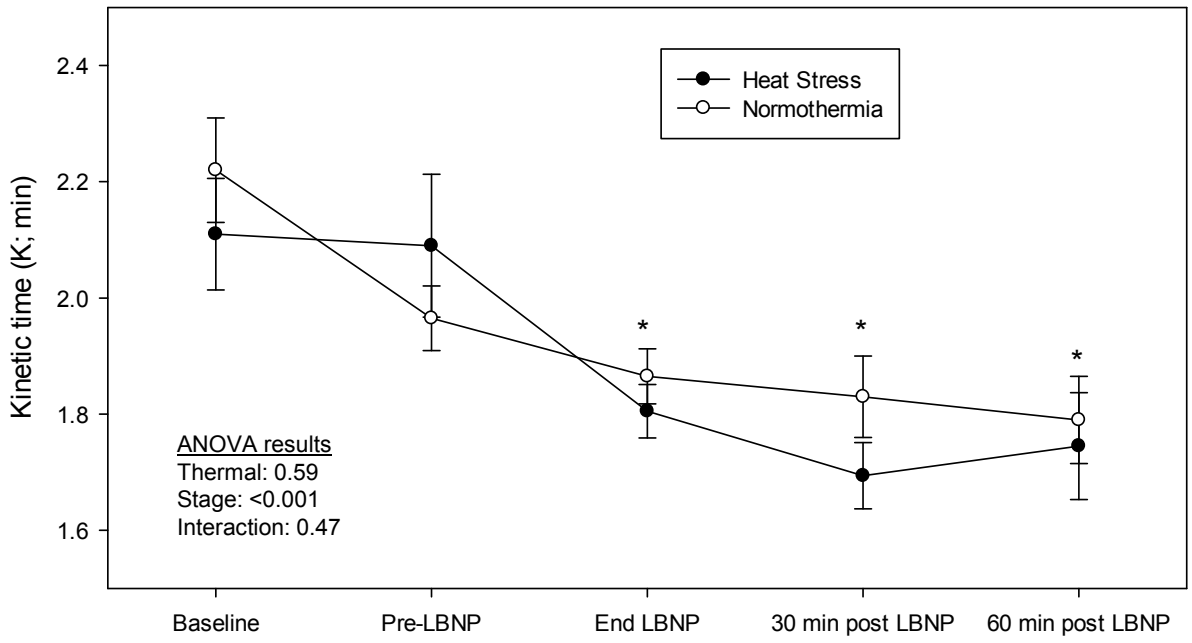

Figure 5: Effect of heat stress and lower-body negative pressure (LBNP) on thromboelastography kinetic time (K). * significant difference for the indicated stage relative to baseline, regardless of thermal condition.

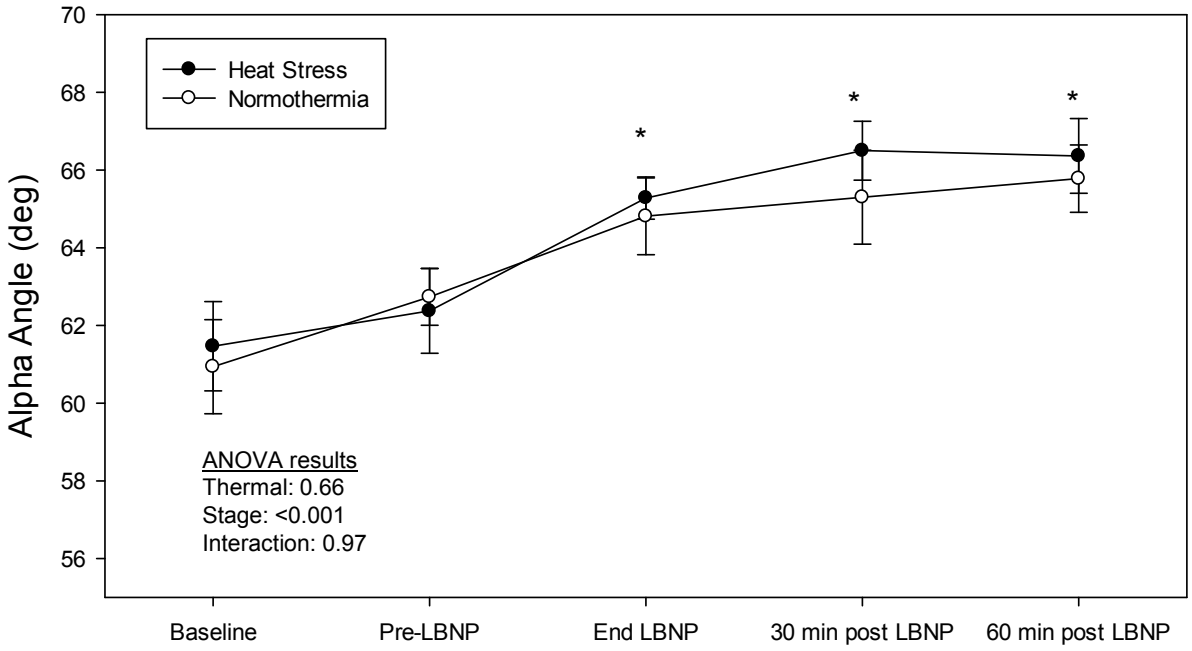

Figure 6: Effect of heat stress and lower-body negative pressure (LBNP) on thromboelastography α angle. * significant difference for the indicated stage relative to baseline, regardless of thermal condition.

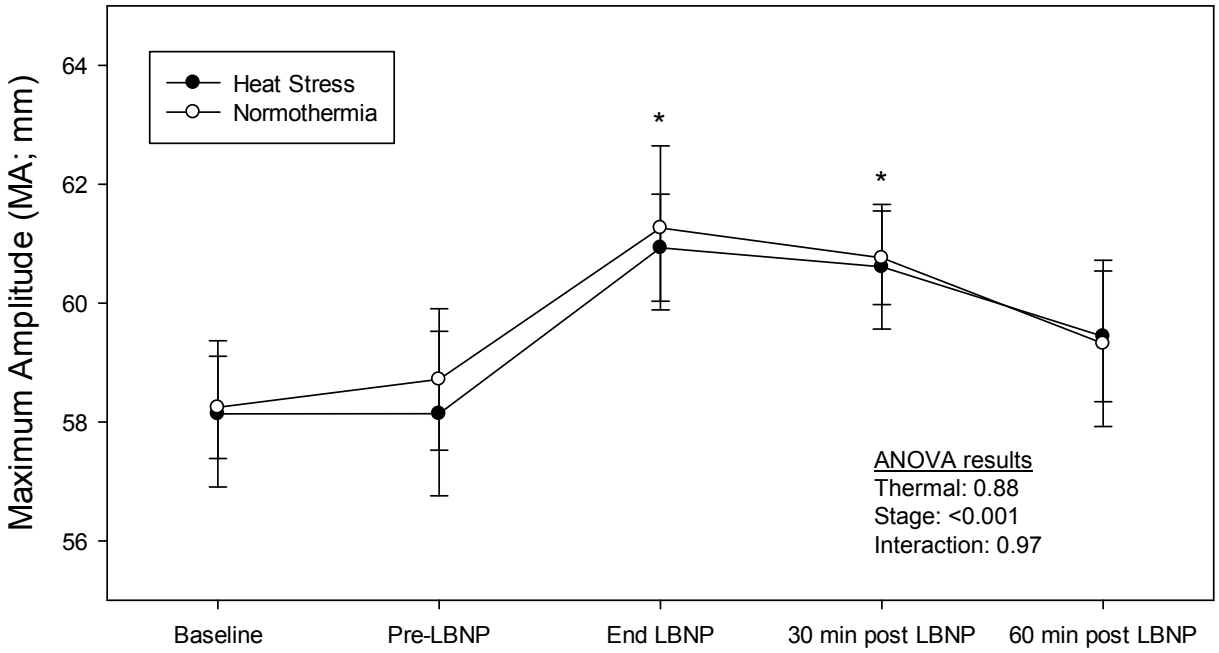

Figure 7: Effect of heat stress and lower-body negative pressure (LBNP) on thromboelastography maximum amplitude (MA). * significant difference for the indicated stage relative to baseline, regardless of thermal condition.

Planned work during the ensuing 12 months:

We will complete data collection for the current protocol in early May 2013. The plasma-based assays will then be run by laboratory personnel under the direction of Andre Cap, MD at the USAISR. We anticipate having results from those assays by the end of summer 2013. We will work with the three external companies (Flashback Technologies, Reflectance Medical, and Body Media), also with a goal of having those results in hand by the end of summer 2013. Finally, we will initiate the 2nd Aim of the protocol (labeled Aim 1B in the original application). The objective of this aim is to evaluate the interactive effects exercise-induced hyperthermia, dehydration, and simulated hemorrhage on markers of hemostatic function. This protocol is outlined in Figure 8 below.

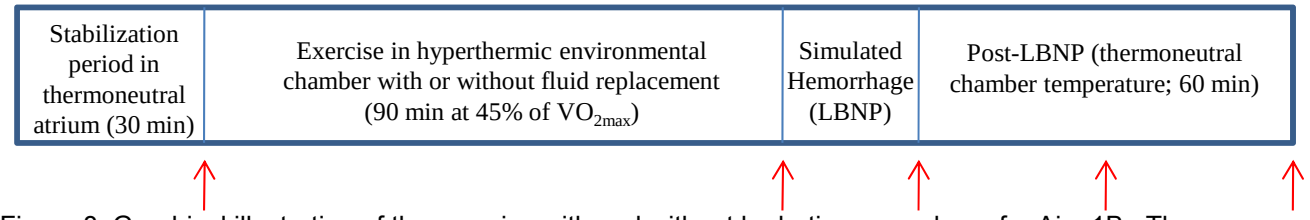

Figure 8: Graphical illustration of the exercise with and without hydration procedures for Aim 1B. The arrows indicate when blood will be drawn for evaluation of markers of hemostatic function. Thermal and hemodynamic variables will be continuously obtained. LBNP: lower body negative pressure; VO_{2max}: maximal oxygen uptake.

Key Research Accomplishments

- Obtained IRB approval from the University of Texas Southwestern Medical Center and Texas Health Presbyterian Hospital Dallas, as well as ORP approval from the US Army
- Acquired thromboelastography (TEG) units and became proficient their use.
- Completed both limbs of data collection on 10 of the proposed 12 subjects, with the final 2 subjects scheduled to be completed in early May.
- Identification from preliminary data that passive heat stress does not alter thromboelastography-based markers of hemostasis alone and during simulated hemorrhage.

Reportable Outcomes

- These data will be submitted for presentation at the 2013 Military Health System Research Symposium.
- Upon completion of data collection and analysis, one or two manuscripts will be written covering the findings of this work.
- Eric Rivas, MS, is a minority doctoral student at Texas Woman's University who will conduct his doctoral dissertation studies in Dr. Crandall's laboratory. Mr. Rivas is funded in part through grant dollars originating from this project. Importantly, this project is giving Mr. Rivas valuable laboratory experience in preparation for his doctoral studies.

Conclusions

The obtained data, should they remain consistent upon completion of the protocol, indicate that passive heat stress (as would be experienced by a gunner on a vehicle, a sniper, or any other condition where a soldier is exposed to the sun and thus is passively heated) does not alter whole-blood based markers of hemostatic function alone or following a simulated hemorrhagic challenge. Assuming these findings will be consistent with plasma-based markers of hemostasis, these data will be beneficial to those who treat the hemorrhaging soldier in the field by informing them that no modification in hemostasis control is needed when an injured soldier is heat stressed. Perhaps just as valuable as these data, will be the evaluation of the results from devices, such as the Flashback CipherSensor, in predicting the extent of a hemorrhagic injury in the heat stressed soldier. Should it be found that heat stress modifies the algorithms used to predict hemorrhagic status by these devices, this would be critical information to implement into the devices before they are used in combat situations under conditions of elevated environmental temperatures.

References

None

Appendices

None

Supporting data

See data in the body of the text above.